ably around 400 ug/mL. By analogy, avidin may be replaced in these methods by other poly-valent biotin-specific receptors such as streptavidin.

[0163] Experiments were conducted to demonstrate the benefit of using poly-avidin capture layers on carbon ink electrodes and/or the two-step immobilization procedures of the invention. These experiments used screen printed carbon ink electrodes that were patterned on a plastic substrate. The working electrodes had an exposed circular area of about 3 mm^2 that was defined by a patterned dielectric layer that was screen printed over the carbon ink electrodes. The substrate also comprised at least one additional carbon ink electrode for use as a counter electrode. Reagents were immobilized by depositing (using a Bio-Dot dispensor) small volumes (200-300 nL) of a solution comprising the reagent onto the exposed electrode area (the solution being confined to the exposed electrode area by the dielectric layer) and allowing the solution to dry on the electrode. Poly-avidin was prepared by combining the appropriate amounts of avidin and biotin-BSA and incubating for 15 minutes. After the immobilization and/or washing steps (as described below), the substrate was either mated with a multi-well plate top so as to form the bottom surface of a well of multi-well plate or it was mated using a gasket made of double stick tape to a plastic sheet so as to form the bottom surface of a flow cell of an assay cartridge. The electrode surfaces were contacted with a buffered solution comprising tripropylamine (MSD Assay Buffer, MSD) by adding the buffer to a well of a multi-well plate or by introducing the buffer into the flow cell. ECL was induced by applying a voltage between the working and counter electrode (a ramp of 2-5 V over 3 seconds). ECL was measured by taking an image of the substrate using a cooled CCD camera.

[0164] Electrodes were coated with either avidin (by treating with 200 nL of a 75 ug/mL solution of avidin) or with poly-avidin (by treating with 200 nL of a solution containing 75 ug/mL avidin and 3.1 ug/mL biotin-labeled BSA and allowing the solutions to dry overnight; the BSA being labeled with a 4-fold excess of biotin-LC-sulfo NHS ester and having an expected ratio of biotins per BSA of roughly 2-3). The substrates were washed with water and the electrodes were then treated with 300 nL of a solution containing 100 ug/mL of an biotin-labeled anti-TSH antibody. The electrodes were washed with water, assembled into a cartridge into which was introduced a solution containing 20 uIU/mL of TSH and 12 ug/mL of an anti-TSH antibody that was labeled with a Sulfo-TAG NHS ester (MSD), an electrochemiluminescent label. The cartridge was incubated for 8 minutes to allow the binding reactions to occur, the substrate was then washed by passing MSD Assay Buffer into the flow cell and ECL was measured. The average emitted electrochemiluminescence intensity from the poly-avidin treated electrode (1652 units) was approximately three times that from the avidin treated electrode (602 units). Without being bound by theory, it is believed that the higher signal on the poly-avidin electrode represents an increased number of binding sites on the poly-avidin treated electrode and/or a reduction in the amount of avidin that washes off the poly-avidin electrode and adsorbs on other surfaces of the cartridge (thus competing with binding sites on the electrode).

[0165] In a similar experiment, the direct adsorption of anti-TSH antibody (by treatment of the electrode with a 100 ug/mL solution of an anti-TSH antibody) was compared to immobilization via a poly-avidin layer (as described above except that the poly-avidin solution contained 400 ug/mL avidin and 25 ug/mL biotin-BSA and the biotin-labeled anti-TSH was at a concentration of 100 ug/mL). The results showed that signal obtained using immobilization via poly-avidin (2207) was roughly twice that obtained using direct adsorption (1264). In addition, two step immobilization protocol was found to provide more precise results; the coefficients of variation (CVs) were three times lower when the two step method was employed.

[0166] The poly-avidin layers were further characterized by using avidin that was labeled with an electrochemiluminescent label (on average 0.3 Sulfo-TAG NHS labels per protein). The electrodes were treated with one of three solutions: (i) 75 ug/mL avidin, (ii) 75 ug/mL avidin and 25 ug/mL BSA or (iii) 75 ug/mL avidin and 25 ug/mL biotin-BSA. All the solutions contained 0.0035% Triton X-100. The electrodes were washed with water, immersed in MSD Assay Buffer and ECL was measured. The electrode treated with all the components of poly-avidin (avidin and biotin-BSA) gave an ECL signal (150981) that was roughly twice that observed for avidin alone (85235) or avidin with unlabeled BSA (65570), demonstrating that cross-linking was required for the improved performance of poly-avidin. It was also observed that the intensity of ECL was much more evenly distributed across the electrode for the poly-avidin electrodes than for the other electrodes.

[0167] In a different experiment the labeled and immobilized avidin or poly-avidin layers were i) not washed or ii) exposed to a solution containing BSA for 2 hours and then extensively washed with phosphate buffered saline. In this experiment, the avidin concentration was 0.5 mg/mL, the ratio of avidin to biotin-BSA was 16:1 and the labeled avidin was mixed with unlabeled avidin (at a 1:100 ratio) to reduce the overall signals. The experiment was carried out on both non-treated electrodes and electrodes that were treated with an oxygen plasma. Table 5, below, shows that the use of poly-avidin substantially reduced the loss of avidin from the surface after extensive washes and exposure to protein-containing solutions.

TABLE 5

	Unmodified Electrodes				Plasma-Treated Electrodes			
	Avidin		Poly-Avidin		Avidin		Poly-Avidin	
	Signal	% Left	Signal	% Left	Signal	% Left	Signal	% Left
No Wash	21,107		26,618		10,871		18,512	
Wash	9,545	45	18,845	71	3,332	31	14,024	76

[0168] After immobilizing assay reagents on surfaces for use in solid phase assays (e.g., by applying solutions com-